



Go faster. Go further. Go global.

GlobalFiler™

Powered by 6-Dye™

*Alabama's Internal Validation
of the
GlobalFiler STR Kit
for Forensic Casework*



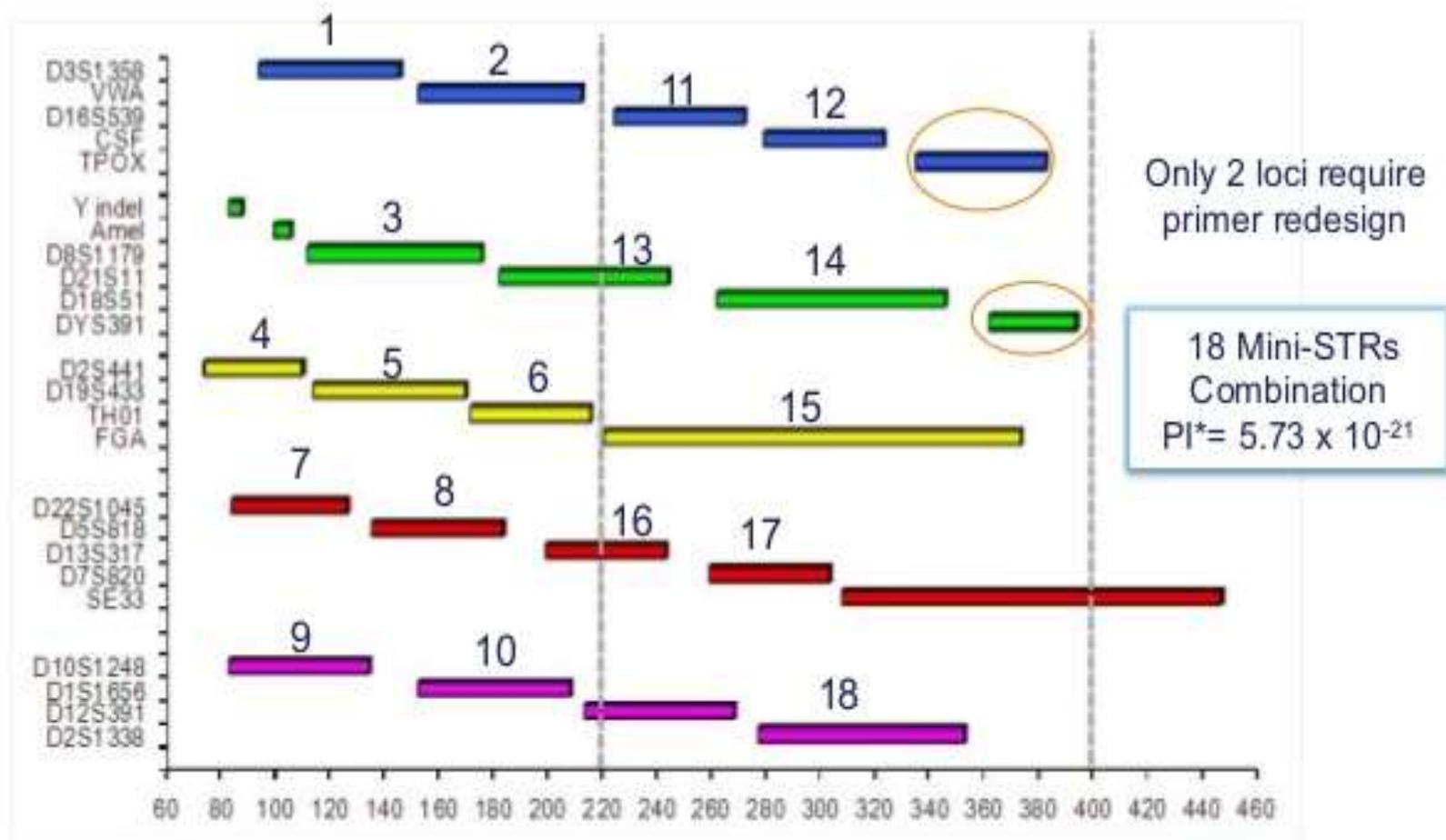
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Alabama Department of Forensic Sciences

Mobile Regional Laboratory

The GlobalFiler™ Kits: 6-Dye Configuration



GlobalFiler™ Kit
 10 Mini-STRs
 $PI^* = 9.2 \times 10^{-12}$

MiniFiler™ Kit
 8 Mini-STRs
 $PI^* = 1.05 \times 10^{-10}$

97% of alleles in allelic ladder <400bp

FBI's Quality Assurance Standards for Forensic DNA Testing Laboratories

8.3.1 For Internal Validation Studies:

- a. Have internal validation studies been documented and summarized?
- b. Have all internal validation studies conducted on or after July 1, 2009, included, as applicable:



1. Known and non probative evidence samples or mock evidence samples?

Yes No N/A

2. Reproducibility and precision?

Yes No N/A

3. Sensitivity and stochastic studies?

Yes No N/A

4. Mixture studies?

Yes No N/A

5. Contamination assessment?

Yes No N/A

Internal Validation Studies - Background

Current Kit (Casework):	Identifiler
Detection Platform:	3130 Genetic Analyzer
3130 Analytical Threshold:	75 rfu



Test Kit:	GlobalFiler
Detection Platform:	3500 Genetic Analyzer (8 capillary)
3500 Analytical Threshold:	<i>175 rfu – starting point</i>

First and Foremost...

PCR Cycle Number and Post-Amplification Conditions Evaluation

•27, 28, and 29 amplification cycles were evaluated:

Manufacturer's recommendation (29 cycles)

1. a dilution series of the 007 human male DNA control
2. a range of mixed DNA samples consisting of varying amounts of the 007 and 9947A human DNA controls

CRITERIA EVALUATED

- overall peak heights
- peak morphology
- *presence of artifacts*
- *ability to detect the minor component of a mixture*
- the overall success rate of each sample

First and Foremost...

PCR Cycle Number and Post-Amplification Conditions Evaluation

- *Post-Amplification conditions were evaluated* on the above samples using:
 1. 1 μ l and 2 μ l of amplified product
 2. 15 and 20 second injection times

CRITERIA EVALUATED

- *overall peak heights*
- peak morphology
- *presence of artifacts*
- *ability to detect the minor component of a mixture*
- the overall success rate of each sample

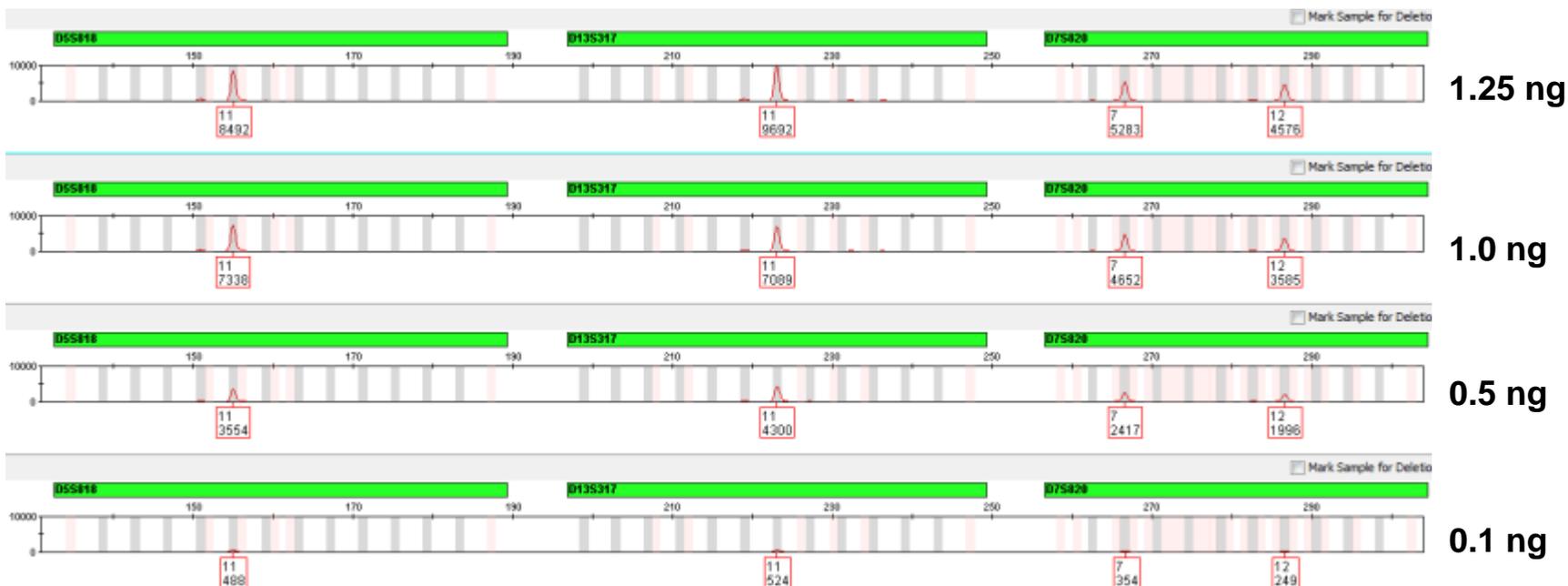
PCR Cycle Number and Post-Amplification Conditions Evaluation

Sensitivity Studies

The following amounts of human DNA control 007 were tested:

1.5, 1.25, 1.0, 0.5, 0.1, 0.05 and 0.01 ng

- each sample was amplified for 27, 28, and 29 PCR cycles, *in triplicate*
- 1 μ l and 2 μ l of amplified product were injected for 15 secs and 20 secs



Sensitivity Studies-Alleles Detected

Average Number of 007 Alleles Detected (43 unique alleles)

Sample	27 cycles				28 cycles				29 cycles			
	1 μ l 15 secs	1 μ l 20 secs	2 μ l 15 secs	2 μ l 20 secs	1 μ l 15 secs	1 μ l 20 secs	2 μ l 15 secs	2 μ l 20 secs	1 μ l 15 secs	1 μ l 20 secs	2 μ l 15 secs	2 μ l 20 secs
1.5 ng	43	43	43	43	43	43	43	43	43	43	43	43
1.25 ng	43	43	43	43	43	43	43	43	43	43	43	43
1.0 ng	43	43	43	43	43	43	43	43	43	43	43	43
0.5 ng	43	43	43	43	43	43	43	43	43	43	43	43
0.1 ng	10	17.3	10	20.3	34	39.3	32.3	40	41.3	42	41.3	42.3
0.05 ng	0.3	1	0.7	1.3	9.7	16.3	8.7	16.3	21.3	29.7	24.3	32
0.01 ng	-	-	-	-	-	-	-	-	0.3	1.7	-	2

Sensitivity Studies-Alleles Detected

Average Number of 007 Alleles Detected (43 unique alleles)

Sample	27 cycles				28 cycles				29 cycles			
	1 μ l 15 secs	1 μ l 20 secs	2 μ l 15 secs	2 μ l 20 secs	1 μ l 15 secs	1 μ l 20 secs	2 μ l 15 secs	2 μ l 20 secs	1 μ l 15 secs	1 μ l 20 secs	2 μ l 15 secs	2 μ l 20 secs
0.1 ng	10	17.3	10	20.3	34	39.3	32.3	40	41.3	42	41.3	42.3
0.05 ng	0.3	1	0.7	1.3	9.7	16.3	8.7	16.3	21.3	29.7	24.3	32

Goal: *Maximize the data and Minimize the artifacts*

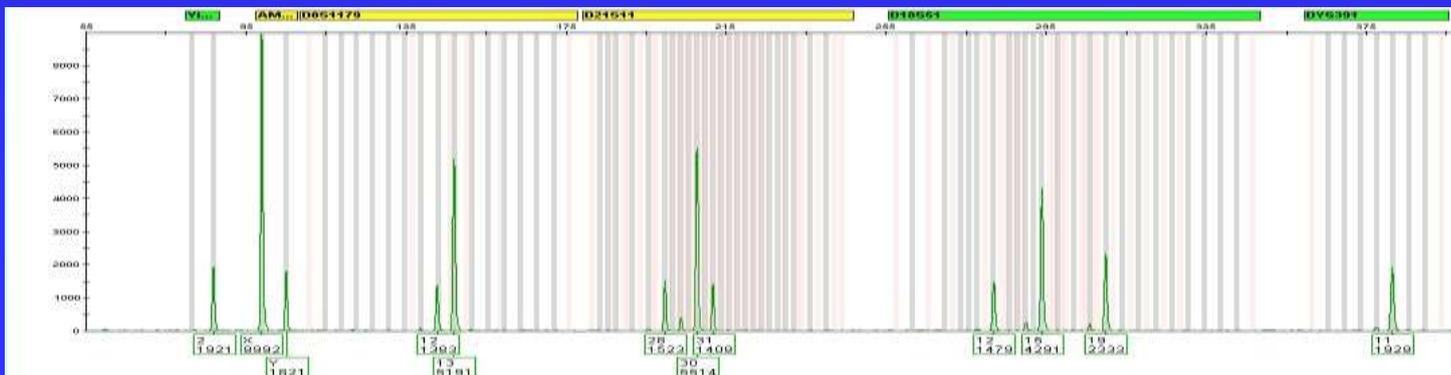
PCR Cycle Number and Post-Amplification Conditions Evaluation

Mixture Studies

The human DNA controls 9947A and 007 were mixed in the following ratios:

1:0, 99:1, 79:1, 59:1, 39:1, 19:1, 9:1, 4:1, 3:2, 1:1, 2:3, 1:4, 1:9, 1:19, 1:39, 1:59, 1:79, 1:99, and 0:1

- *one (1) nanogram* of each mixture was amplified for 27, 28, and 29 PCR cycles, in triplicate
- 1 μ l and 2 μ l of amplified product were injected for 15 secs and 20 secs on the 3500 Genetic Analyzer



Mixture Studies-Alleles Detected

Average Number of 007 Alleles Detected (26 unique alleles)

MIXTURE 007:9947A	27 cycles				28 cycles				29 cycles			
	1 µl 15 secs	1 µl 20 secs	2 µl 15 secs	2 µl 20 secs	1 µl 15 secs	1 µl 20 secs	2 µl 15 secs	2 µl 20 secs	1 µl 15 secs	1 µl 20 secs	2 µl 15 secs	2 µl 20 secs
99:1	26	26	26	26	26	26	26	26	26	26	26	26
79:1	26	26	26	26	26	26	26	26	26	26	26	26
59:1	26	26	26	26	26	26	26	26	26	26	26	26
39:1	26	26	26	26	26	26	26	26	26	26	26	26
19:1	26	26	26	26	26	26	26	26	26	26	26	26
9:1	26	26	26	26	26	26	26	26	26	26	26	26
4:1	26	26	26	26	26	26	26	26	26	26	26	26
3:2	26	26	26	26	26	26	26	26	26	26	26	26
1:1	26	26	26	26	26	26	26	26	26	26	26	26
2:3	26	26	26	26	26	26	26	26	26	26	26	26
1:4	22.3	25	23	25	26	26	26	26	26	26	26	26
1:9	9.3	14	6.7	15	19	23	19.3	22.7	25.3	25.3	25.3	25.7
1:19	0.7	2.3	0.7	2.7	8	12.3	9	13	17.7	19	18	20.7
1:39	0	0	0	0	1.3	1.7	1.3	2.3	6.7	1.3	8.3	10.7
1:59	0	0	0	0	0	0.3	0	0.3	3.7	4.7	4.3	6.3
1:79	0	0	0	0	0.3	0.3	0.3	0.3	1.7	3.3	2	3.7
1:99	0	0	0	0	0	0	0	0	0.7	1	0.7	1.3

Mixture Studies-Alleles Detected

Average Number of 007 Alleles Detected (26 unique alleles)

MIXTURE 007:9947A	27 cycles				28 cycles				29 cycles			
	1 μ l 15 secs	1 μ l 20 secs	2 μ l 15 secs	2 μ l 20 secs	1 μ l 15 secs	1 μ l 20 secs	2 μ l 15 secs	2 μ l 20 secs	1 μ l 15 secs	1 μ l 20 secs	2 μ l 15 secs	2 μ l 20 secs
1:4	22.3	25	23	25	26	26	26	26	26	26	26	26
1:9	9.3	14	6.7	15	19	23	19.3	22.7	25.3	25.3	25.3	25.7
1:19	0.7	2.3	0.7	2.7	8	12.3	9	13	17.7	19	18	20.7
1:39	0	0	0	0	1.3	1.7	1.3	2.3	6.7	1.3	8.3	10.7
1:59	0	0	0	0	0	0.3	0	0.3	3.7	4.7	4.3	6.3
1:79	0	0	0	0	0.3	0.3	0.3	0.3	1.7	3.3	2	3.7
1:99	0	0	0	0	0	0	0	0	0.7	1	0.7	1.3

Goal: *Maximize* the data and *Minimize* the artifacts

Mixture Studies-Artifacts Detected

Number of Artifacts Detected (57 samples tested)

	27 cycles				28 cycles				29 cycles			
	1 μ l 15 secs	1 μ l 20 secs	2 μ l 15 secs	2 μ l 20 secs	1 μ l 15 secs	1 μ l 20 secs	2 μ l 15 secs	2 μ l 20 secs	1 μ l 15 secs	1 μ l 20 secs	2 μ l 15 secs	2 μ l 20 secs
Pull-up	-	-	-	-	-	-	-	-	1	7	4	10
Background	-	-	-	-	-	-	-	-	1	2	1	5
Hi-ST	-	-	-	-	-	2	3	3	9	8	6	7
Hi-ST %	-	-	-	-	-	12.4%	12.6%	12.4%	22.8%	23.2%	17.9%	22.2%

Hi-ST mainly at FGA – 11.55% cutoff

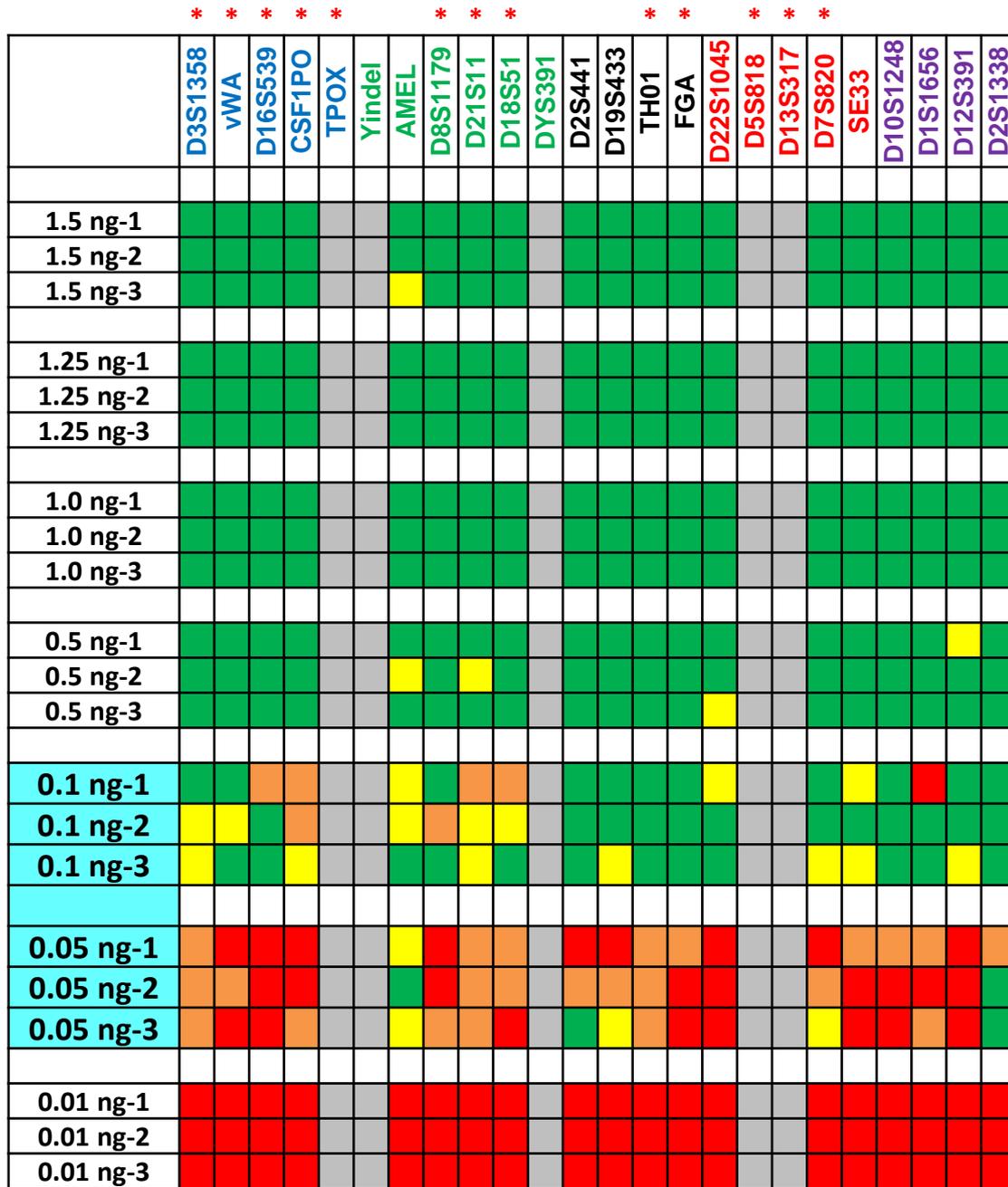
Results of the PCR Cycle Number and Post-Amplification Conditions Evaluation

- Optimal Number of PCR cycles: 28
- Post-Amplification Conditions:
 - 2 μ l of amplified product with 20 second injections

28 PCR cycles with 2 μ l of amplified product injected for 20 seconds maximized the data obtained while minimizing artifacts that would complicate downstream interpretations (i.e. Mixtures)

These analysis parameters were then used to assess Stochastic Effects (PHR and allele dropout) in the overall sensitivity and mixture studies

PHR Heat Map-Stochastic Effects

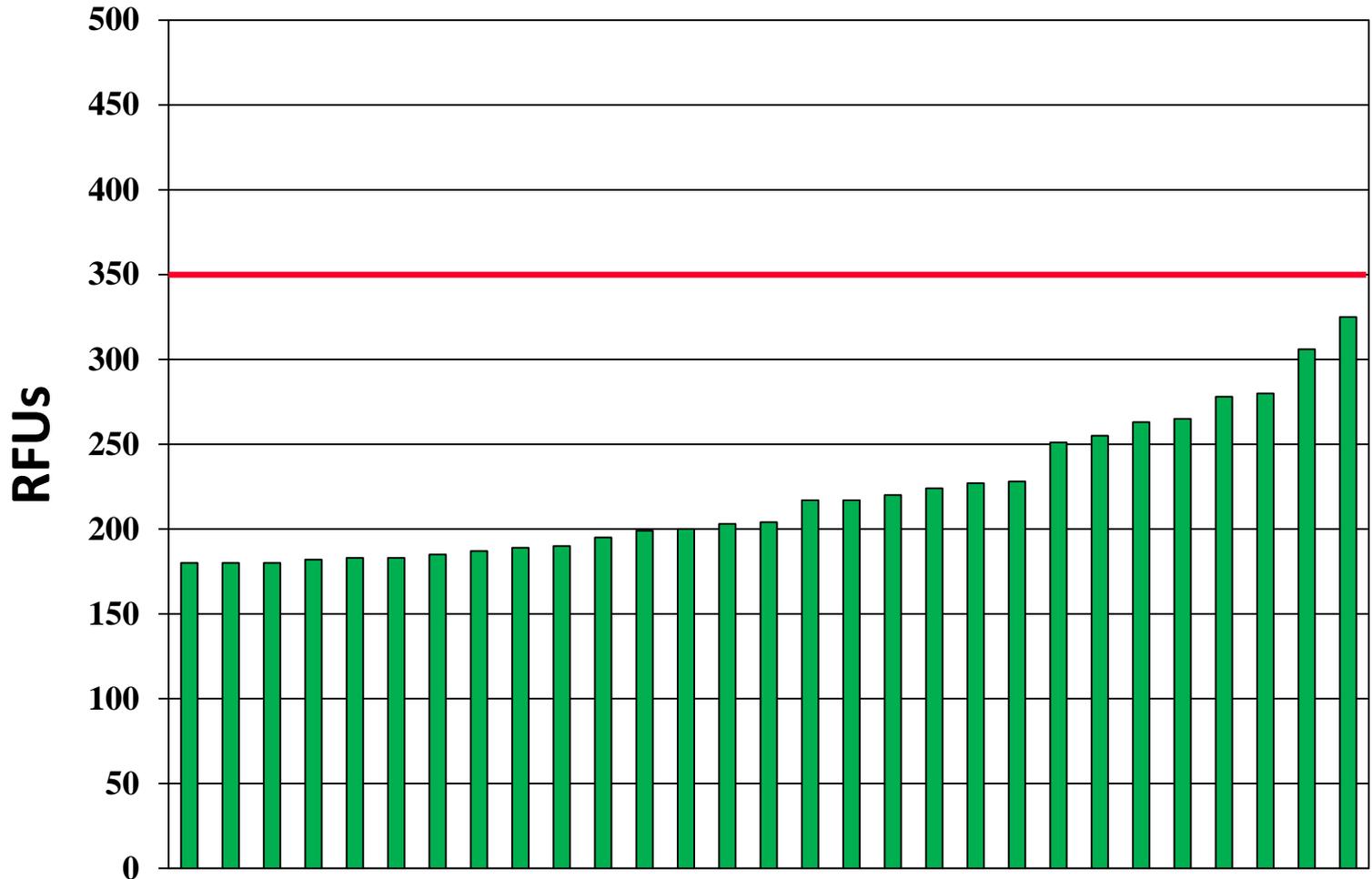


 homozygous locus
 * CODIS core locus

 peak height ratio > 70%
 peak height ratio < 70%
 drop-out of one allele
 drop-out of both alleles

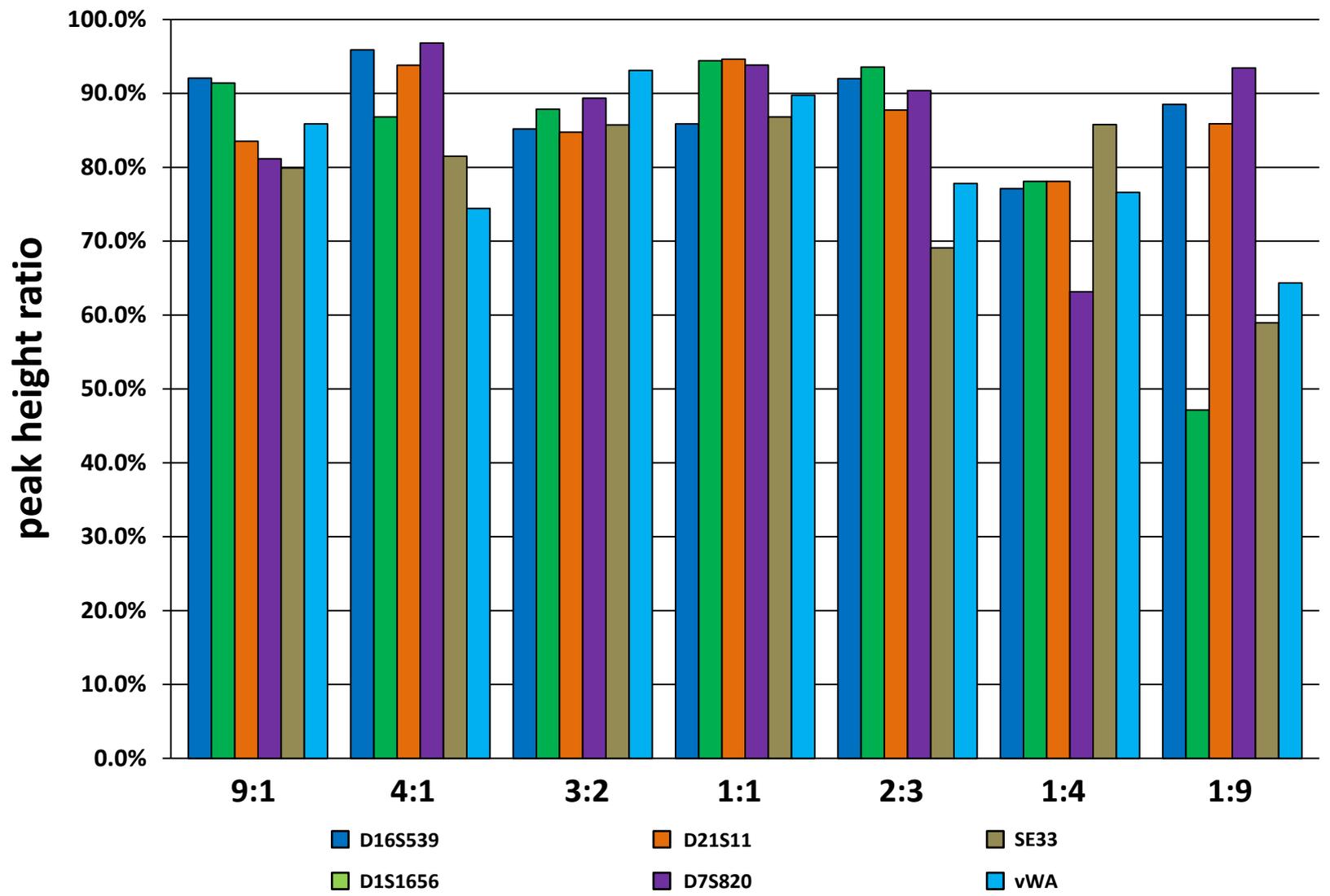
Sensitivity Studies-Allele Dropout

Peak Height of Detected Allele at a Locus with Dropout

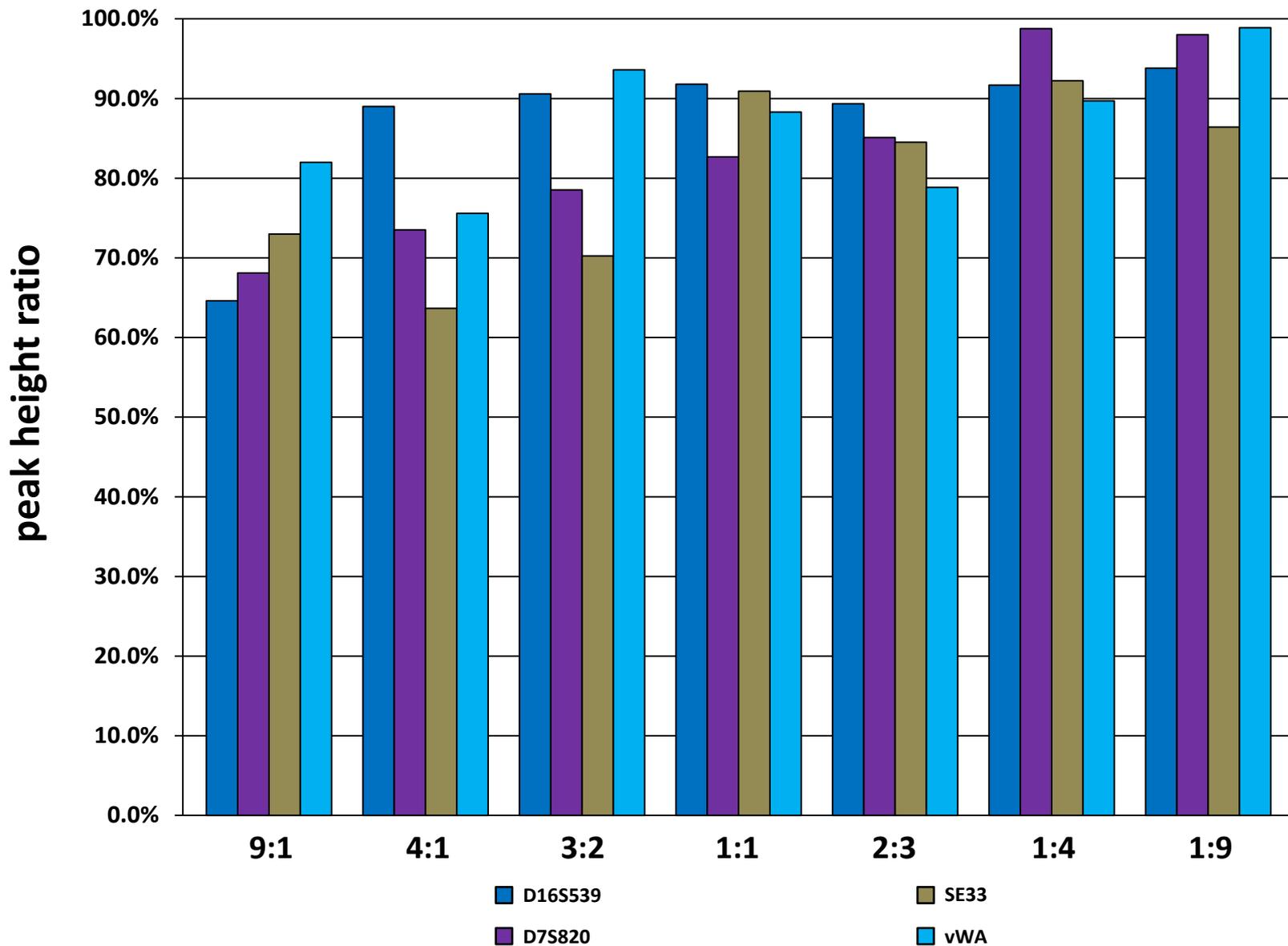


- analytical threshold of 175 RFUs utilized
- 399 total heterozygote loci
- at 84 of the 399 loci all detected alleles were less than 500 RFUs
- at 29 of the 84 loci one allele dropped-out

007 PHR in mixtures (007:9947A)-Stochastic Effects

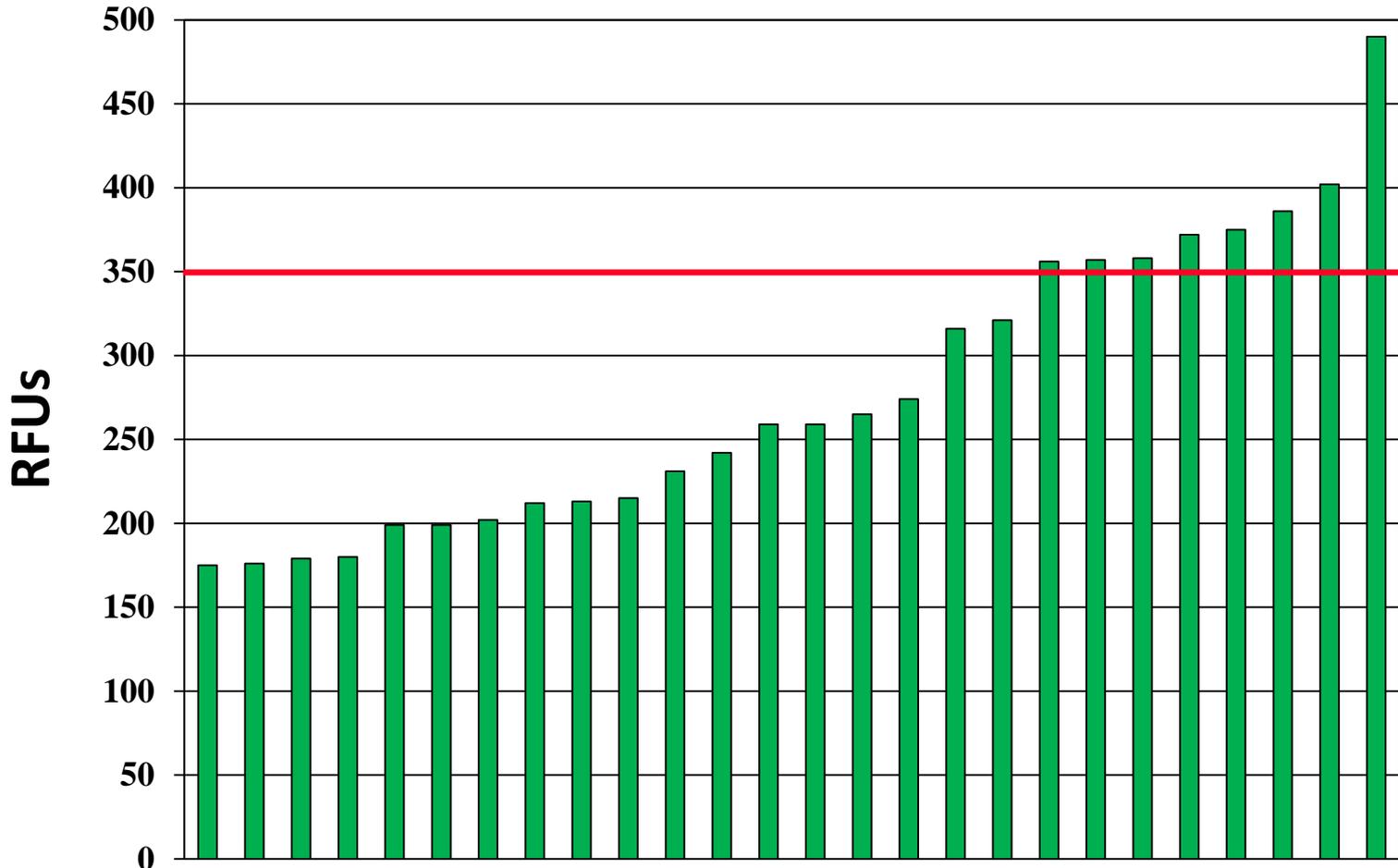


9947A PHR in mixtures (007:9947A)-Stochastic Effects



Mixtures Studies-AAllele Dropout

Peak Height of Detected Allele at a Locus with Dropout



- analytical threshold of 175 RFUs utilized
- 306 total loci with non-overlapping heterozygote alleles
- at 53 of the 306 loci all detected alleles were less than 500 RFUs
- at 26 of the 53 loci one allele dropped-out



Known and Non-Probative Evidence Samples

Previously extracted non-probative evidence samples, which mimic other sample types routinely encountered in forensic casework, were tested with the GlobalFiler STR Kit. The results obtained using GlobalFiler were compared to those previously obtained with the Identifiler STR Kit.

The sample types included in this study included:

- sexual assault samples (VS, condom, bedding, and panties)**
- wearer items**
- cigarette butts**
- bottle/can swabs**
- blood swabs**
- degraded/inhibited samples**
- reference samples (buccal swabs and blood cards)**



Known and Non-Probative Evidence Samples

GlobalFiler:

- tests 24 loci
- 21 loci are autosomal STRs
- *12 loci < 250 bp*

Identifiler:

- tests 16 loci
- 15 loci are autosomal STRs
- *9 loci < 250 bp*

variables:

- input volume differences (*10ul vs 15ul*)
- chemistry differences between the GF and ID kits
- 3500 and 3130 differences
 - injection parameters
 - analysis algorithms

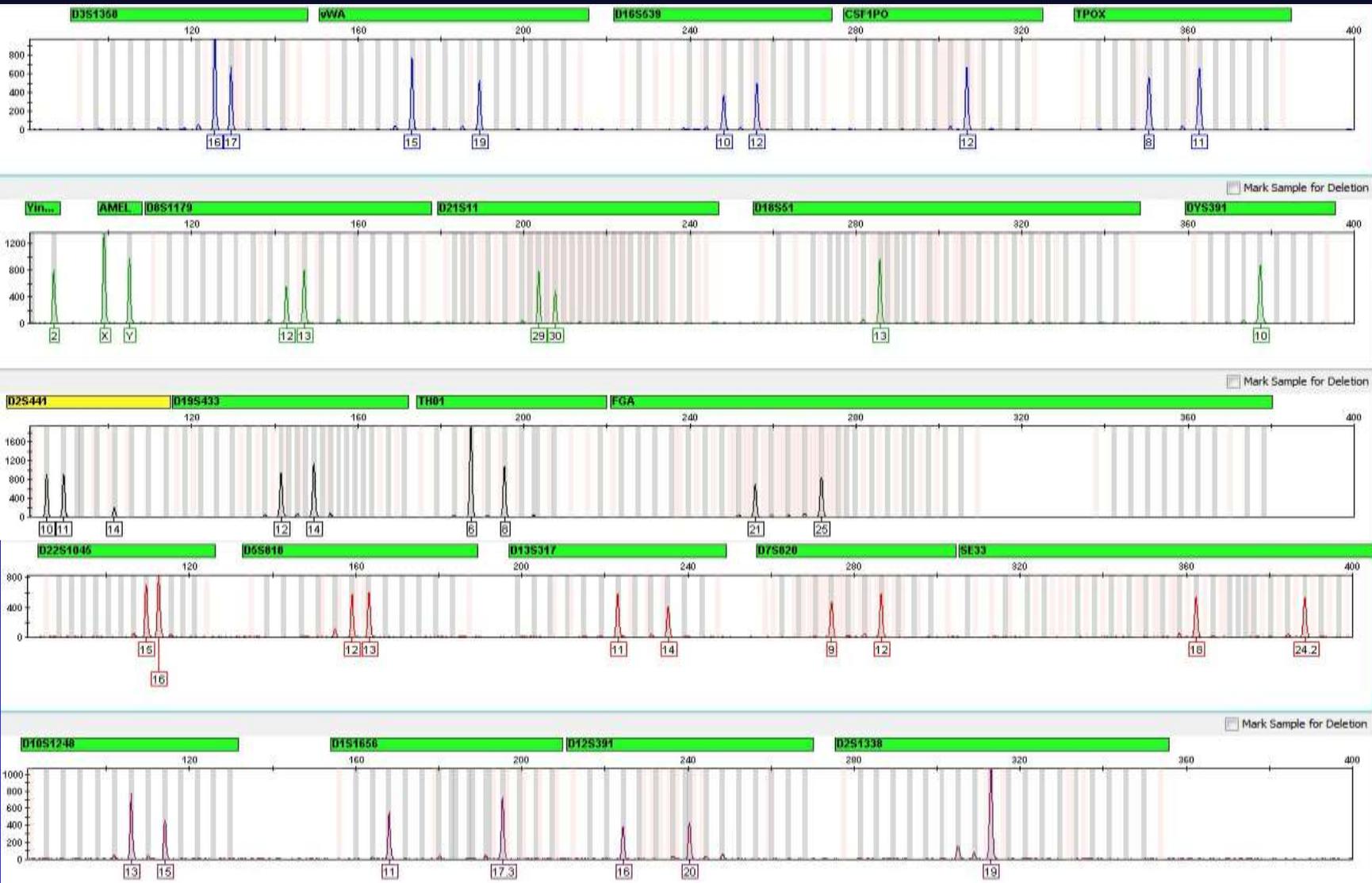


- Great concordance between GlobalFiler and Identifiler
- More information from GlobalFiler due to the additional dye and the number of loci < 250 bp
- GlobalFiler much more effective in overcoming **inhibition and degradation**

1) Semen stain from a quilt

- 0.041 ng/μl
- 0.41 ng amplified with Identifiler
- maximum injection parameters on the 3130 (2 μl/ 9 secs)
- partial male DNA profile
(10 loci of data with 7 of the CODIS core)

GlobalFiler



- **0.62 ng amplified with GlobalFiler**
- **full male DNA profile (13 CODIS core)**



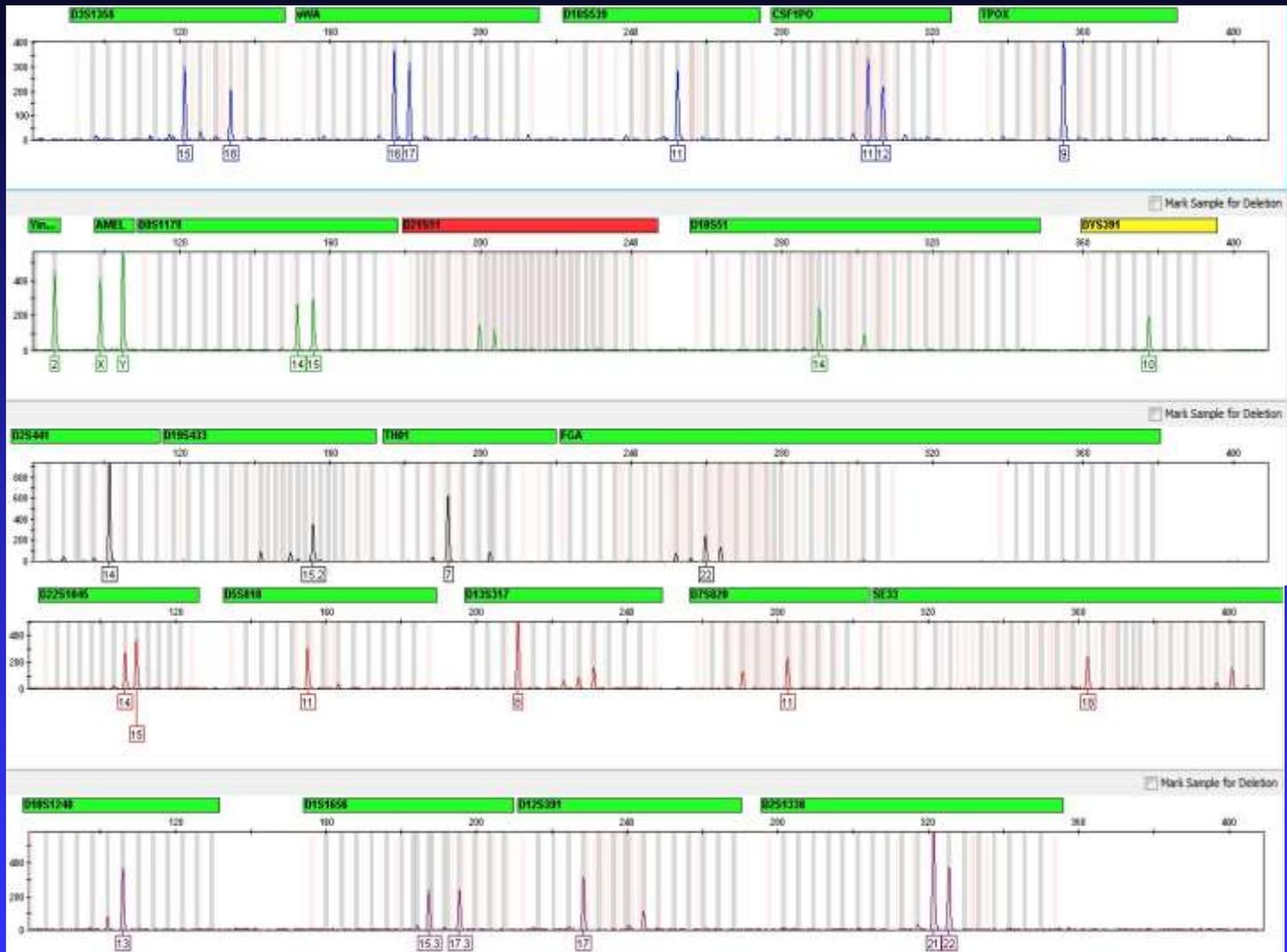
Known and Non-Probative Evidence Samples

2) Semen stain from a pair of panties

- 0.0091 ng/ μ l
- 0.09 ng amplified with Identifiler
- maximum injection parameters on the 3130 (2 μ l/ 9 secs)
- partial male DNA profile
- 12 loci of data with 9 of the CODIS core



GlobalFiler



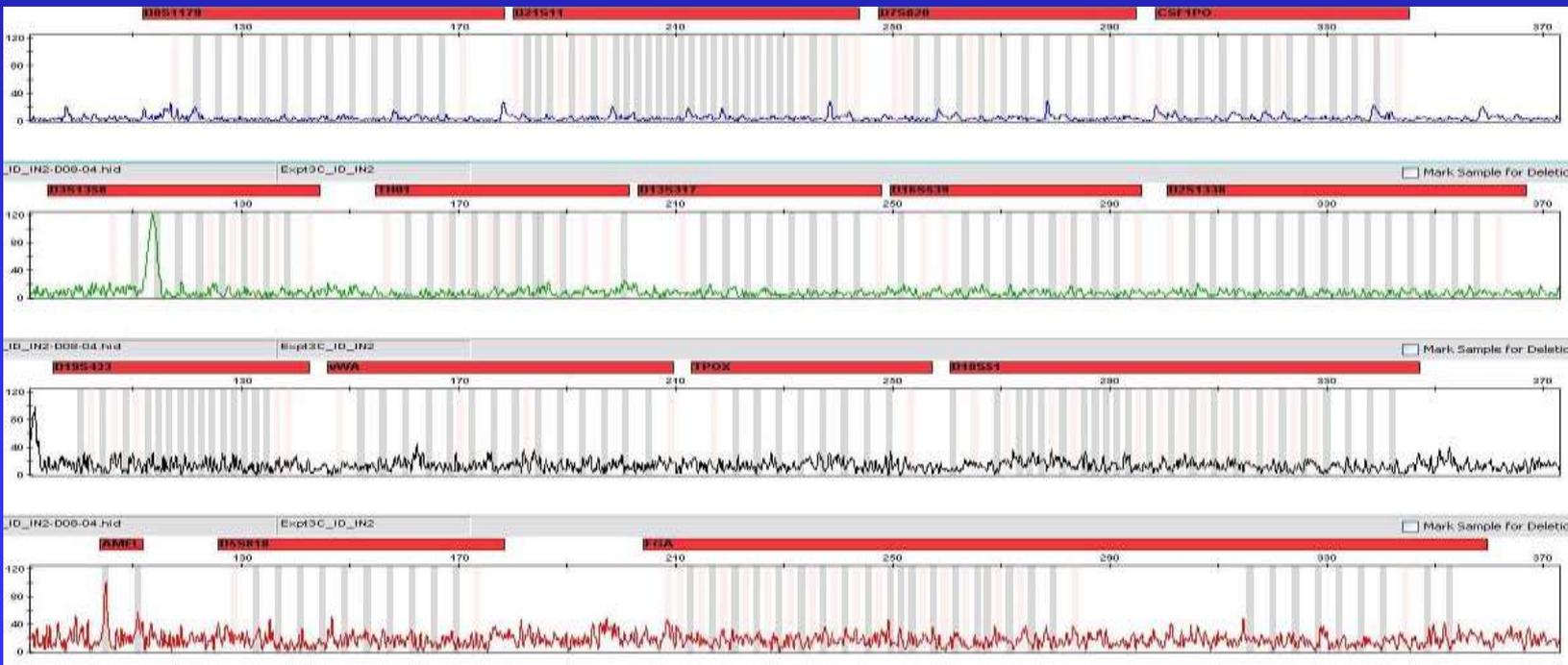
- 0.14 ng amplified with GlobalFiler
- partial male DNA profile
- 23 loci of data and 12 CODIS core



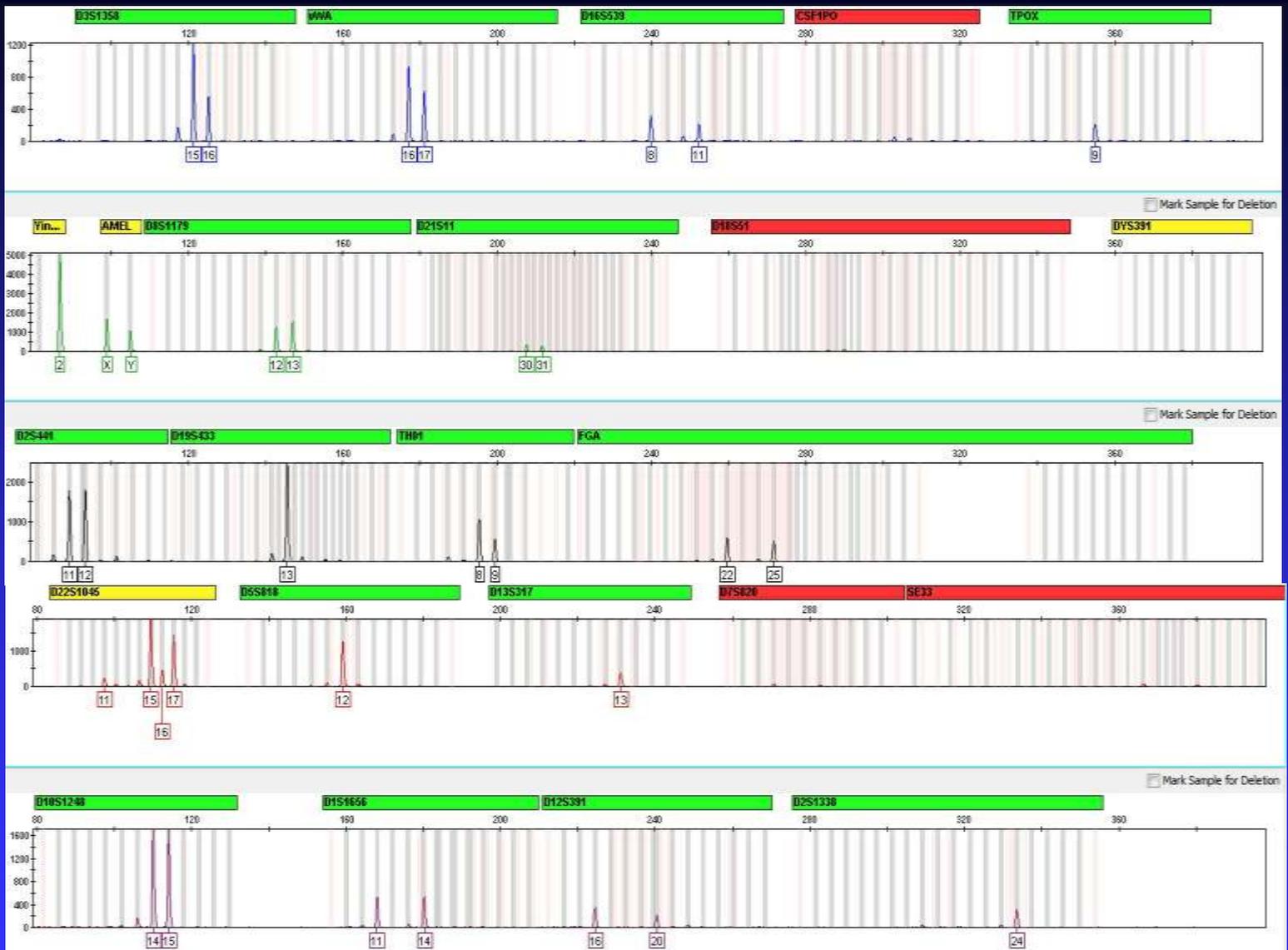
Known and Non-Probative Evidence Samples

3) Swabbing of a concrete block

- 0.17 ng/ μ l
- 1 ng amplified with Identifiler
- standard injection on the 3500 (1 μ l/15 secs)
- *no results*
- *inhibition suspected due to extract color*

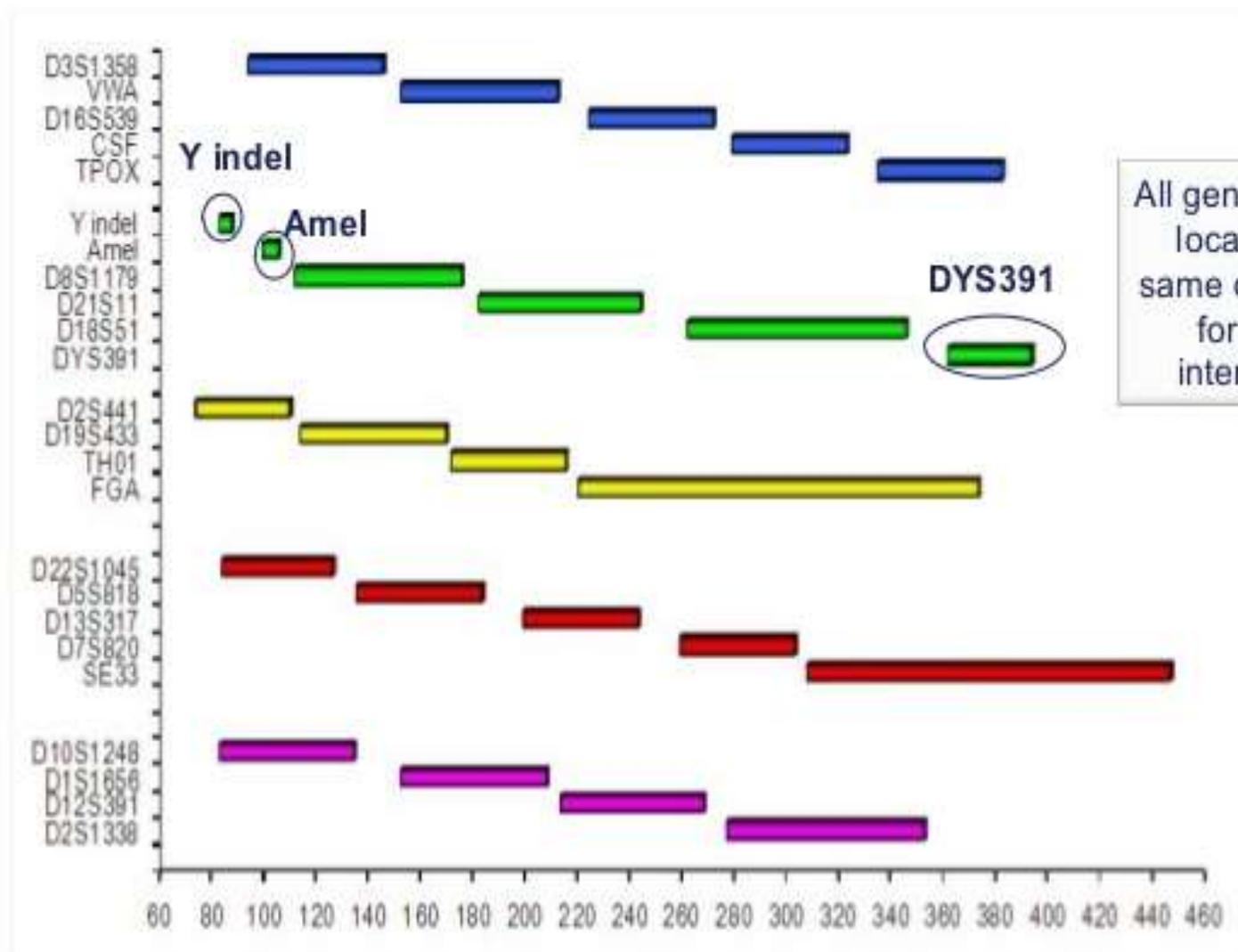


GlobalFiler



- 1 ng amplified with GlobalFiler
- **partial DNA profile (19 loci of data; 10 CODIS core)**

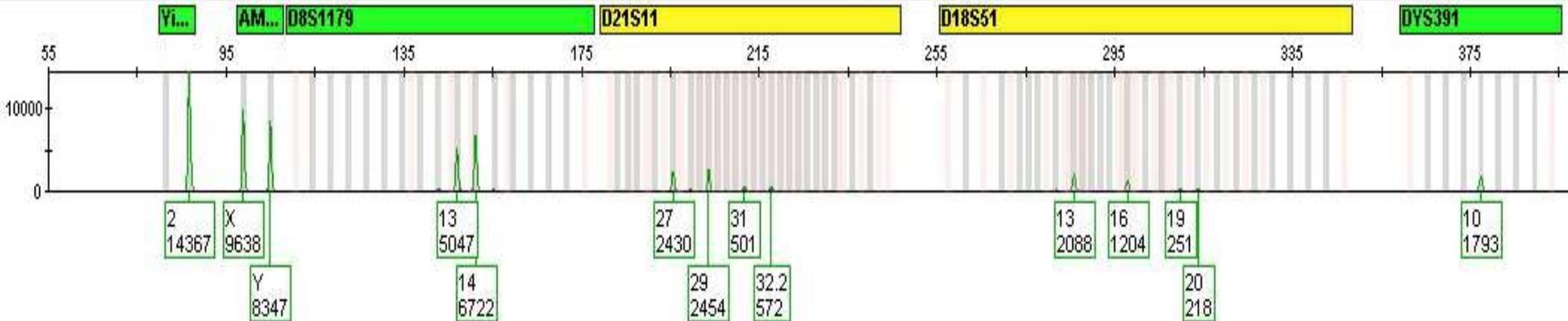
The GlobalFiler™ Kits: Configuration



All gender markers located in the same dye channel for ease of interpretation

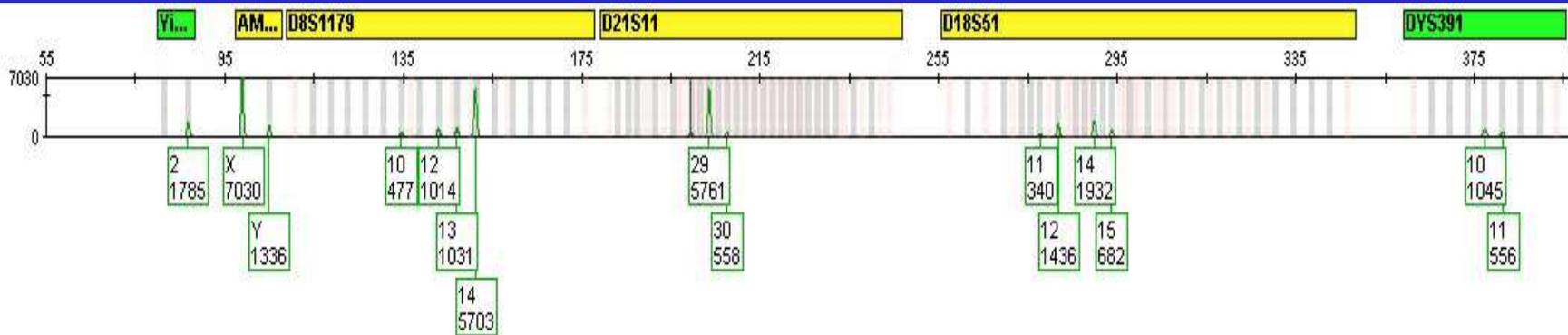
Mixture Studies-Male Gender Marker

DYS201



-mixture containing at least 1 male

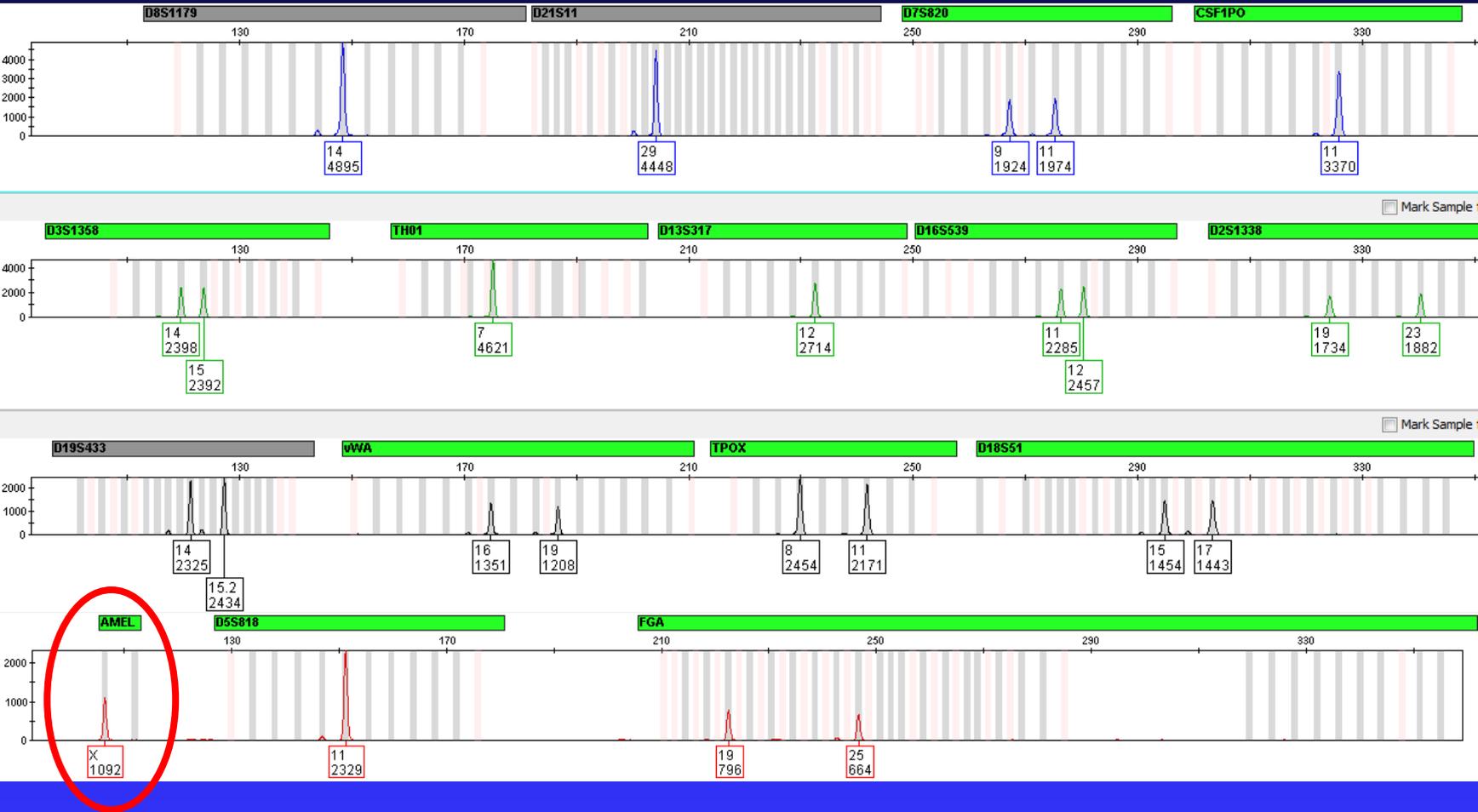
10 allele



-mixture containing at least 2 males

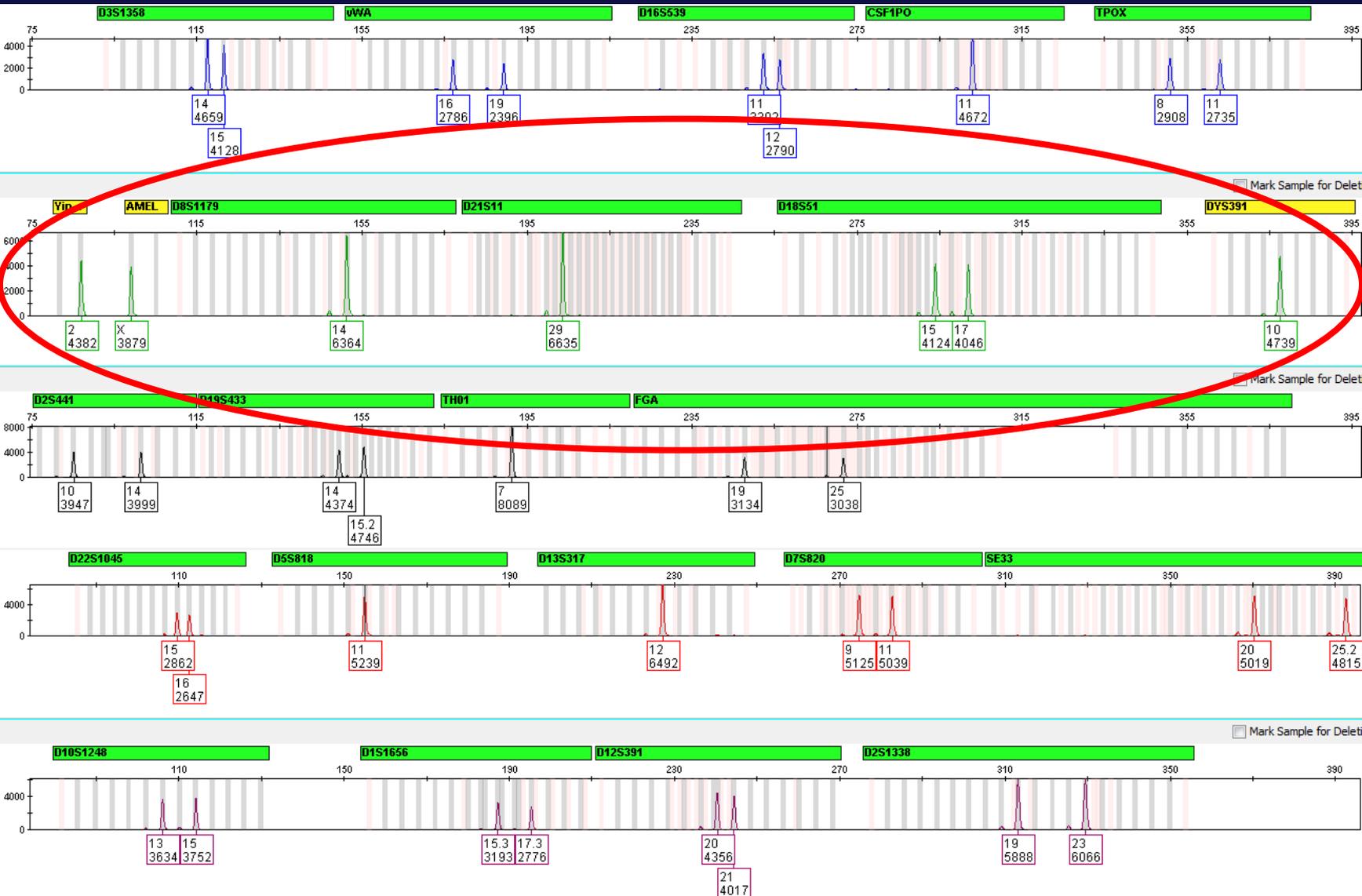
10 and 11 alleles

Male Gender Marker Redundancy-Identifiler

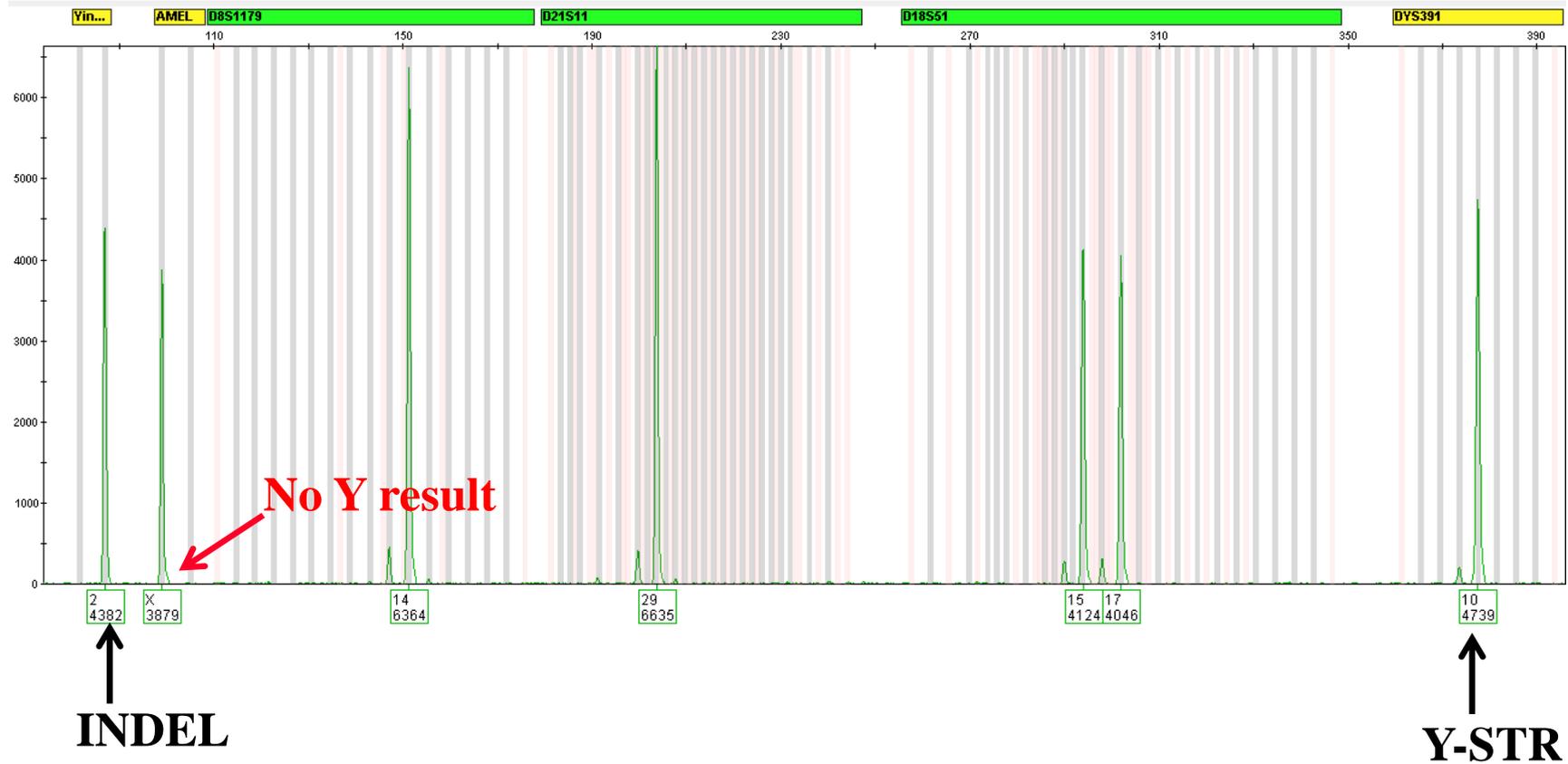


DNA reference sample from a self-identified male

Male Gender Marker Redundancy-GlobalFiler



Male Gender Marker Redundancy-GlobalFiler



Reproducibility, Precision, and Accuracy

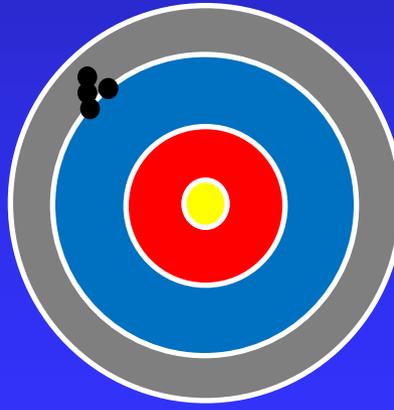
Precision: characterizes the amount of agreement among a series of individual (or repeated) measurements

-**Precision** does not relate to the *accuracy* of the measurements

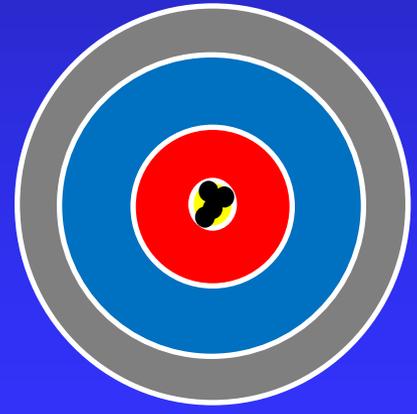
Accuracy: describes how close the measured values are to the true value



**Low Precision
Low Accuracy**



**High Precision
Low Accuracy**



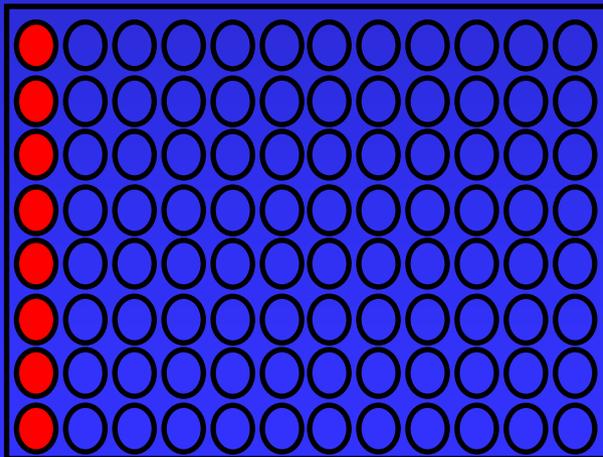
**High Precision
High Accuracy**

Reproducibility and Precision Study Design

GlobalFiler allelic ladder was prepared in one column and injected eight (8) times on the 3500 Genetic Analyzer

-size determinations were conducted on all alleles of the ladder for each injection

-standard deviations in base pair sizing were calculated for each allele in the ladder

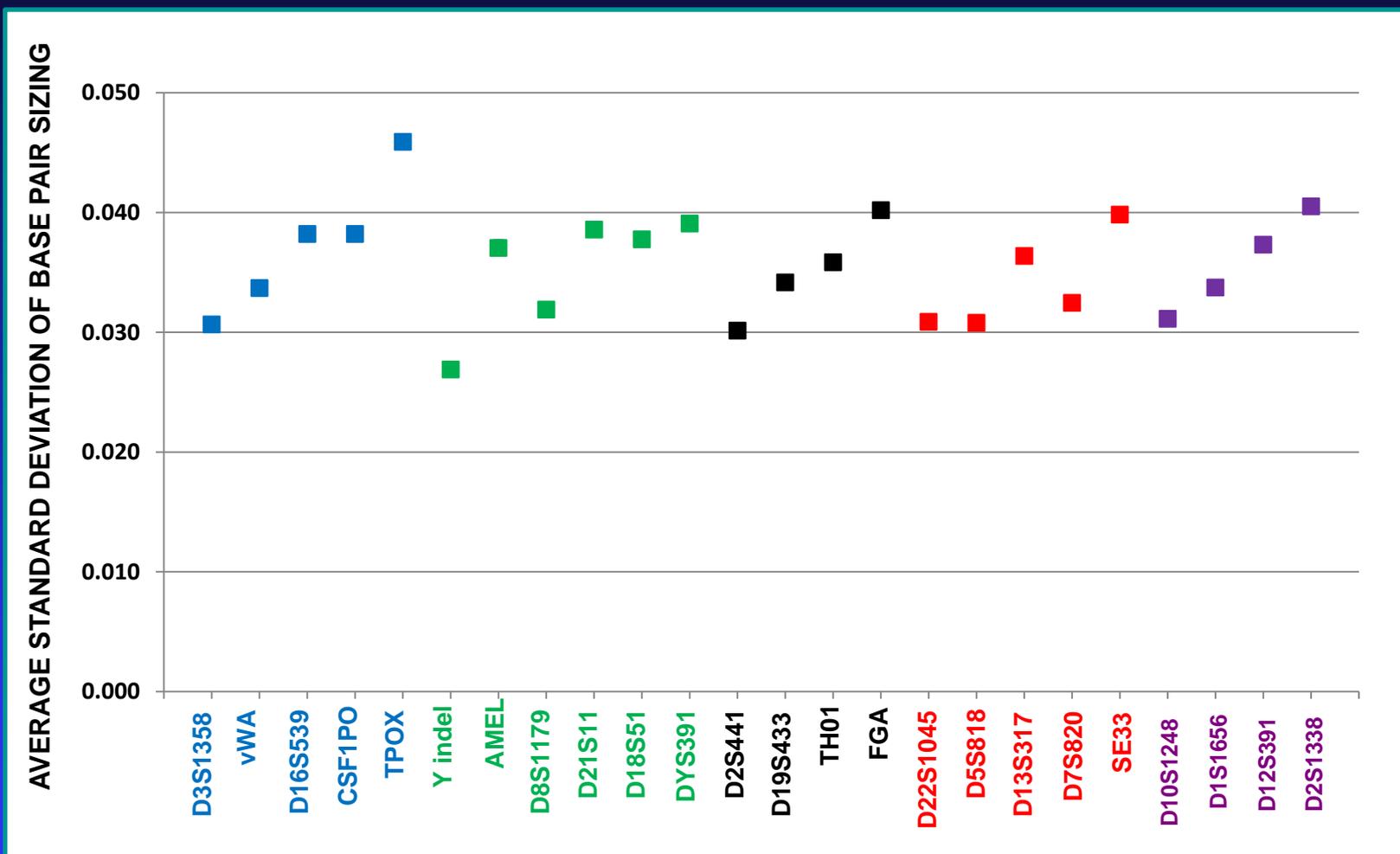


**GlobalFiler allelic ladder – 343 alleles across
24 loci**

↓
2,744 alleles per 8-cap injection

↓
21,952 alleles per run of 8 injections

Reproducibility and Precision of GlobalFiler Allelic Ladder



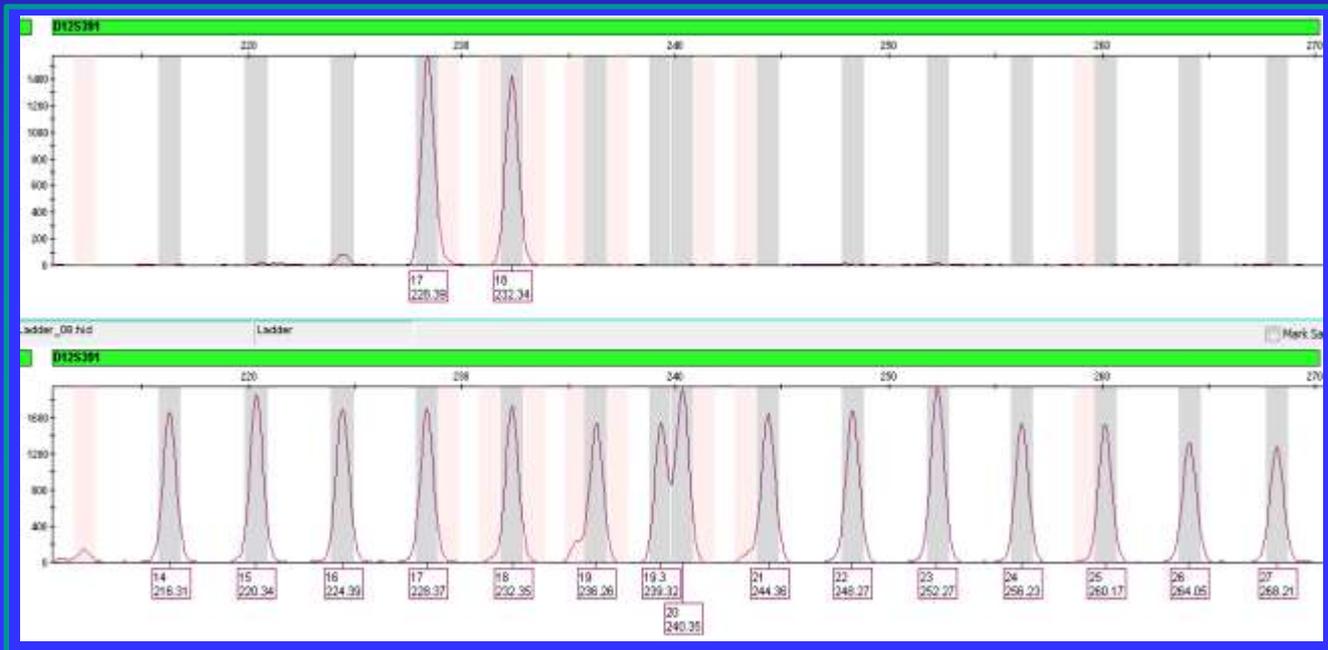
-standard deviation was averaged across all the alleles at each locus

Accuracy Study Design

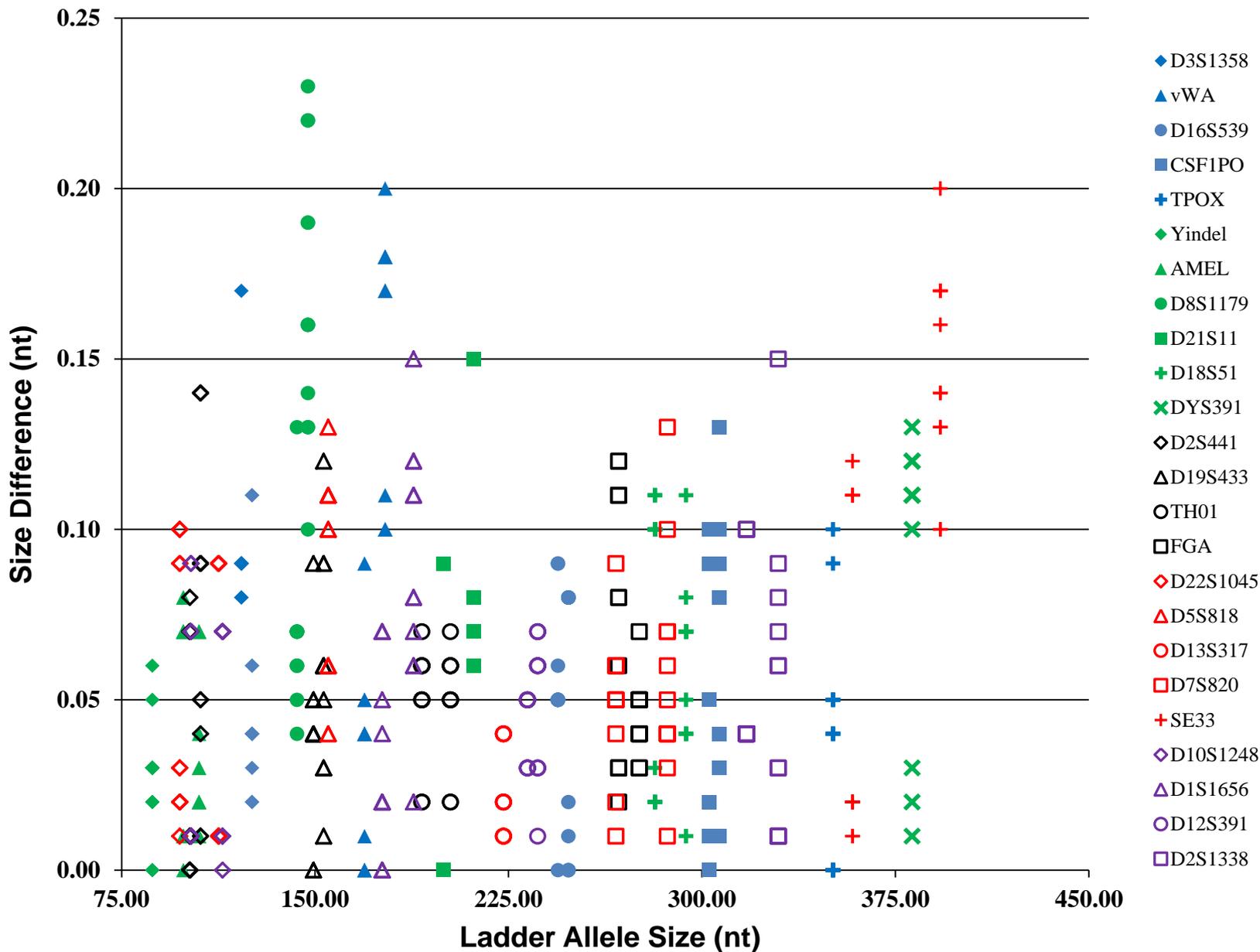
One (1) ng of the 007 Human DNA control was amplified concurrently four times with each amplified product prepared for electrophoresis in triplicate for a total of twelve (12) samples. The twelve (12) samples were injected on three separate days generating data for thirty-six (36) samples across three separate runs.

Accuracy: describes how close the measured values are to the true value

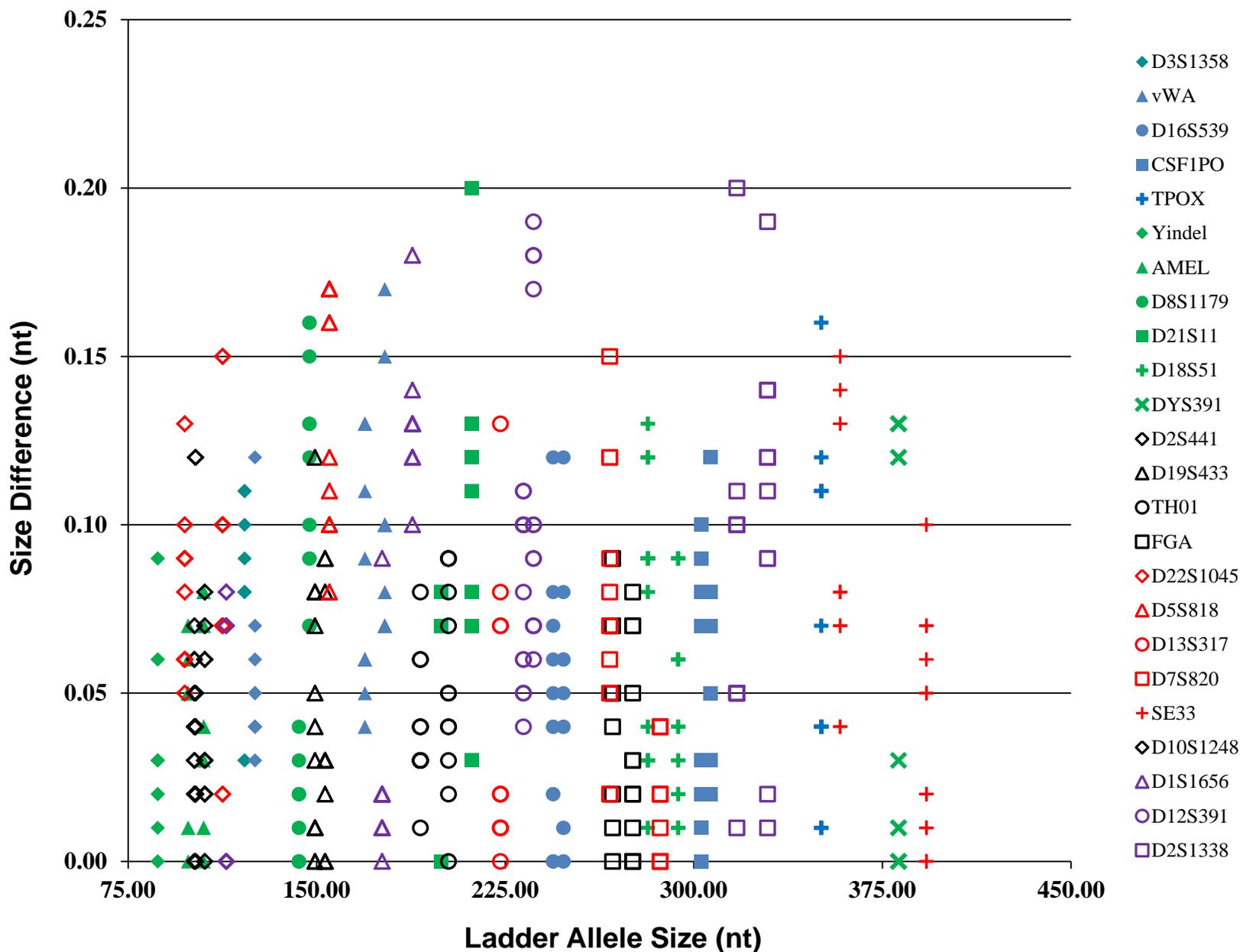
The measurement of each allele in the 007 sample was compared to the measurement of the corresponding allele in the GlobalFiler allelic ladder



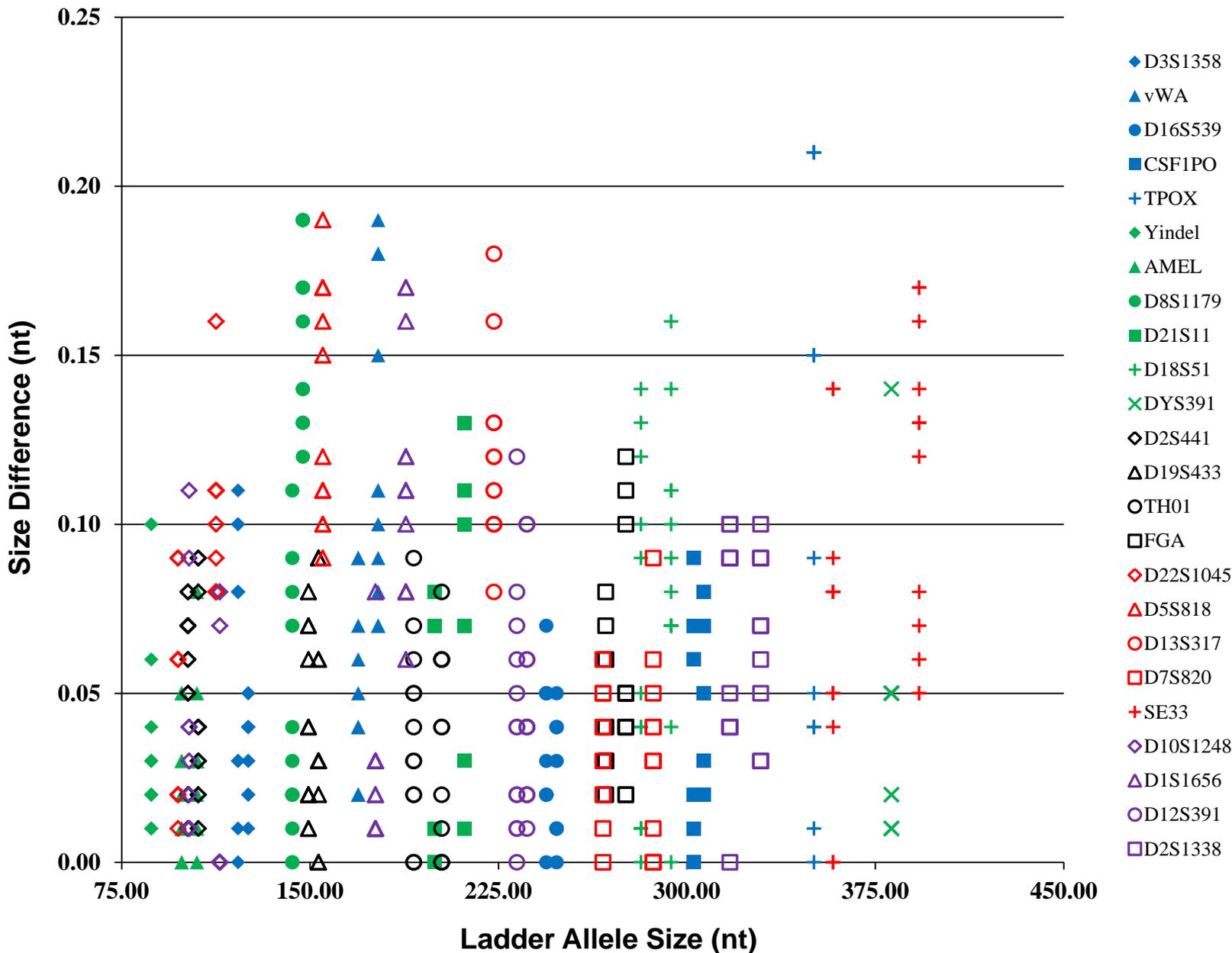
Accuracy Study Day 1



Accuracy Study Day 2



Accuracy Study Day 3



Final Thoughts

Mixture studies:

- nearly complete minor component could be detected in the **1:9** and **9:1** mixture samples
- significant minor component detected in the **1:19** and **19:1** mixture samples.

Sensitivity and stochastic studies:

- nearly complete DNA profiles were obtained from **100 pg** of template
- significant data was obtained from as low as **50 pg** of template.

Final Thoughts

Casework samples:

- Great concordance between DNA profiles previously obtained with Identifiler
- GlobalFiler very successful in overcoming both inhibition and degradation.

Reproducibility and precision of GlobalFiler on the ABI 3500:

the standard deviation across multiple allelic ladder injections was **< 0.05 bp**.

Accuracy of the GlobalFiler STR Kit on the ABI 3500:

all alleles detected from the 007 sample across three separate 3500 runs were within **± 0.25 bp** of the allelic ladder measurement.

Acknowledgements

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